Superparamagnetic composite particles for extraction and purification of genomic DNA

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Silica-coated superparamagnetic iron oxide nanoparticles (SPION) are widely used for a number of biomedical applications [1, 2], including extraction and purification of genomic DNA from biological samples. The method is based on selective adsorption of DNA on γ-Fe_2O_3/SiO_2 silica surface followed by a magnetic separation [3]. Highly pure and concentrated sample of desorpted DNA can be used for real-time polymerase chain reaction (RT-PCR) analysis in clinical diagnostics. In this work, we represent a facile synthesis of γ-Fe_2O_3/SiO_2 composite particles using cheap and environmentally acceptable inorganic chemicals. The synthesis is based on a combined hydrolysis of Fe^{2+} and Fe^{3+} salts with ammonia water solution followed by a hydrolysis of Na_2SiO_3 with hydrochloric acid in the presence of the obtained Fe_3O_4 nanoparticles [1]. The final stage is an oxidation of Fe_3O_4/SiO_2 (35 wt.% SiO_2) into γ-Fe_2O_3/SiO_2 using 9 % H_2O_2 water solution since γ-Fe_2O_3 phase possesses a better thermal and chemical stability. The γ-Fe_2O_3/SiO_2 nanoparticles were dispersed in physiological saline (NaCl 10 $mg \cdot ml^{-1}$) to obtain a water suspension with the solid phase concentration of 40 $mg \cdot ml^{-1}$. The influence of the γ-Fe_2O_3/SiO_2 synthesis conditions on the DNA adsorption, colloidal stability and magnetic properties of the obtained particles were studied.

The diameter of the γ-Fe_2O_3/SiO_2 spherical nanoparticles was estimated by TEM to vary from 40 to 80 nm. The composite particles consist of crystalline γ-Fe_2O_3 grains (8–15 nm) distributed over an amorphous SiO_2 matrix (XRD, TEM, SEM). However, the hydrodynamic size of the nanocomposite, determined by dynamic laser scattering (DLS) measurement was significantly greater (4500–4700 nm). This indicated to a tendency of the particles to form agglomerates in water solution. The powdered nanocomposite shows a superparamagnetic behavior, with the saturation magnetization of about 35–40 $A \cdot m^2 \cdot kg^{-1}$. The specific surface area of the powder was measured by BET method and appeared to be 160–170 $m^2 \cdot g^{-1}$. The presence of functional silica film on the surface of composite particles was confirmed by IR spectroscopy.

In order to estimate the functional properties of the synthesized sorbent suspension, a model DNA isolation kit was proposed. It was composed of γ-Fe_2O_3/SiO_2 particles saline dispersion, lysis solution (guanidine hydrochloride), TE buffer (Tris, EDTA, pH~8,0), ethanol and acetone. The nucleic acids isolation had been performed by a modified Boom method [4]. Human and salmon genomic DNA and clinical samples of human blood serum and saliva were used as model biomaterials. The extraction efficiency was estimated by the quantitative RT-PCR [3] to be 83–94 %. No impurities that can inhibit the PCR were detected during operations. It was found that the purity of the extracted DNA was suitable for the PCR analysis in clinical diagnostics.

Thus, the synthesized γ-Fe_2O_3/SiO_2 nanocomposite suspension meets the basic requirements to sorbent for commercial DNA isolation kit.

References

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